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Risk of Leukemia Following Treatment for Non-Hodgkin's Lymphoma

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Background: **There have been few evaluations of the risk of acute nonlymphocytic leukemia (ANLL) following therapy for non-Hodgkin's lymphoma (NHL). Further, the relationship between cumulative dose of cytotoxic drug, radiation dose to active bone marrow, and the risk of ANLL following NHL have not been well described.** ***Purpose:*** **Our purpose was to examine the risk of ANLL in relationship to all prior treatment for NHL.** ***Methods:*** **Within a cohort study of 11386 2-year survivors of NHL, 35 case patients with secondary ANLL were identified and matched to 140 controls with NHL who did not develop ANLL. The primary eligibility criteria for the cohort included a diagnosis of NHL as a first primary cancer from January 1, 1965, through December 31, 1989; age 18 through 70 years at the time of initial diagnosis; and survival for 2 or more years without the development of a second invasive primary malignancy. Detailed information on chemotherapeutic drugs and radiotherapy was collected for all patients. Standard conditional logistic regression programs were used to estimate the relative risk (RR) of ANLL associated with specific therapies by comparing the exposure histories of ease patients with individually matched controls. Results: Significant excesses of ANLL followed therapy with either prednimustine (RR = 13.4; 95% confidence interval [CI] = 1.1-156;** ***P*** **trend for dose <.05) or regimens containing mechlorethamine and procarbazine (RR = 12.6; 95% CI = 2.0-79;** ***P*** **<.05). Elevated risks of leukemia following therapy with chlorambucil were restricted to patients given cumulative doses of 1300 mg or more (RR = 6.5; 95% Cl = 1.6-26; P<.05). Cyclophosphamide regimens were associated with a small, nonsignificant increased risk of ANLL (RR = 1.8; 95% CI = 0.7-4.9), with most patients receiving relatively low cumulative doses (<20 000 mg). Radiotherapy given at higher doses without alkylating agents was linked to a nonsignificant threefold risk of ANLL compared with lower dose radiation or no radiotherapy.** ***Conclusions:*** **Our results suggest that prednimustine may be a human carcinogen, with a positive dose-response gradient evident for ANLL risk. The low, nonsignificant risk of leukemia associated with cyclophosphamide was reassuring because this drug is commonly used today. Despite the excesses of ANLL associated with specific therapies, secondary leukemia remains a rare occurrence following NHL. Of 10 000 NHL patients treated for 6 months with selected regimens including low cumulative doses of cyclophosphamide and followed for 10 years, an excess of four leukemias might be expected. [J Natl Cancer Inst 86:1450-1457, 1994]**

Treatment-related acute nonlymphocytic leukemia (ANLL) is recognized as an important complication of cancer therapy *(1).* Few studies, however, have quantified the risk of ANLL associated with dose of cytotoxic drugs or radiation used to treat cancer *(2-8).* Moreover, investigations of the carcinogenicity of

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See "Notes'' section following 'Refererees."

therapies used to treat non-Hodgkin's lymphoma (NHL) have been largely limited to global assessments of the relative risk (RR) of ANLL, with estimates ranging from 1 to 1000 based on small numbers of cases *(9-11)*. Very few studies have provided estimates of the risk of ANLL associated with specific chemotherapy regimens or individual cytotoxic agents that are commonly used to treat NHL *(12,13)*. To explore these issues further, we studied secondary ANLL among NHL patients in a collaborative international investigation.

Patients and Methods

Study Patients

A case-control study of secondary ANLL was conducted within a cohort of 11 386 2-year survivors of NHL reported to three cancer registries (the State Health Registry of Iowa, The Ontario Cancer Registry, and The Swedish Cancer Register) and the affiliated tumor registries of The Netherlands Cancer Institute in Amsterdam and The Dr. Daniel den Hoed Cancer center in Rotterdam. A description of all second cancers among long-term survivors in a portion of this cohort was published previously *(14)*. Eligibility criteria for the cohort included a diagnosis of NHL as a first primary cancer during the period from January 1, 1965, through December 31, 1989 (for Ontario, the period ended on December 31, 1987); age 18 through 70 years at the time of initial diagnosis; and survival of 2 or more years without the development of a second invasive primary malignancy. All patients who received initial management at major hospitals were included. Study centers in Ontario consisted of the Princess Margaret Hospital [five of the secondary leukemias in this study were previously reported by Lishner et al. *(11)*] and seven of the eight Regional Cancer Centers. The Regional Cancers Centers are located in Hamilton, Kingston, London, Ottawa, Sudbury, Thunder Bay, Toronto, and Windsor. All centers were included in the survey except the one in Hamilton, where medical records had been destroyed in a fire. In The Netherlands, NHL patients were treated at the affiliated hospital of The Netherlands Cancer Institute (Amsterdam) or the Dr. Daniel den Hoed Cancer Center (Rotterdam). In Iowa, hospitals included those in Des Moines (Iowa Methodist Medical Center [1973-1989] and Mercy Hospital [1973-1989]), Iowa City (The University of Iowa Hospitals and Clinics; 1965-1989), and Cedar Rapids (Mercy Medical Center [1973-1989]). All NHL patients in Sweden who met entry criteria were included in the study population.

For each NHL patient within the cohort, cancer registry incidence data were searched to identify subsequent diagnoses of ANLL *(15-18)*. Mortality files available in several centers were examined to ascertain deaths among NHL patients due to any type of anemia (including refractory anemia) or other bleed disorders. In addition, files of pathology and hematology departments were searched for subsequent diagnoses of malignant hematopoietic disorders. These latter sources disclosed no additional cases of ANLL.

For reported second ANLLs, an independent evaluation of available peripheral blood smears, bone marrow aspirates, biopsy specimens, and/or accompanying reports was undertaken to ensure that these cases did not represent leukemic progressions of NHL (lymphocytic leukemias). Histopathologic material was reviewed for 33 case patients; bone marrow aspirate and biopsy reports were reviewed for the remaining two. The 35 eligible case patients included 25 with ANLL *(19,20)*, eight with myelodysplastic syndromes *(21)*, and two with myeloproliferative disorders. The clinicopathologic features of two case patients were included in a prior report *(22)*. All 35 nonlymphocytic, malignant hematopoietic disorders are subsequently referred to as ANLL for simplicity of presentation.

For each case patient with secondary ANLL, four controls were chosen by random sampling from the defined NHL cohort. Matching factors included cancer registry (or institute in The Netherlands), sex, race (Iowa only), age at NHL diagnosis, calendar year of diagnosis of NHL, and length of follow-up (without a second primary cancer) at least as long as the interval between the case patient's diagnosis of NHL and subsequent ANLL.

Data Abstraction: Quantificaaion of Cytotoxic Drug and Radiation Exposure

For each case patient and control, standardized abstract forms were used to collect demographic and medical record data including information on all NHL therapy during the matched time interval. Sources of data included medical centers that provided initial treatment, local hospitals, radiotherapy facilities, and offices of private physicians. Information on dose and duration of administration were abstracted for all alkylating agents and epipodophyllotoxins and for doxorubicin and bleomycin. For other cytotoxic agents, abstracted data were limited to data and duration of administration. For 96% of patients (94% case patients and 96% controls), information on cumulative dose of chemotherapeutic agents was available from medical records. For the remaining patients, dose was estimated on the basis of duration of therapy (six patients) or imputed from the median dose (one patient) given to other subjects.

Detailed radiotherapy records for each patient were used to calculate radiation dose to 17 partitions of active bone marrow *(23)*. Standard radiotherapy depth dose data were used to calculate the absorbed dose to active bone marrow in a treated volume. In regions outside radiation beams, the dose to active bone marrow was calculated by measurement its water phantoms *(24)*. Doses to individual bone marrow partitions were then weighted and summed to yield one overall mean bone marrow dose. Radiotherapy was primarily administered by megavoltage. Fields were typically located in the abdomen or pelvis or in both (34%), chest PIUS abdomen or pelvis (24%), head and neck only (20%), and chest only (16%). Total body irradiation was rarely used (4%).

Statistical Analysis

Standard conditional logistic regression programs were used to estimate the RR of ANLL associated with specific therapies by comparing the exposure history of the case patient with that of individually matched controls *(25,26)*. Most patients had received either radiotherapy, chemotherapy, or both. Thus, evaluations of the risk of ANLL due to alkylating agents are relative to patients treated with radiotherapy alone, surgical resection of involved tissue, or nonalkylating agent chemotherapy. For analysis, patients were grouped according to total chemotherapy history, baaed on administered alkylating agents. Patients were considered exposed if they received cytotoxic drugs for 1 or more months. Comparisons between treatment groups were baaed on likelihood ratio tests. Two-sided *P* values and 95% confidence intervals (CIs) were computed.

Estimates of the dose-response relationship between specific drugs and risk of ANLL were calculated by dividing patients into three groups on the basis of tertiles of cumulative dose administered to controls within that treatment category. The RR was then calculated between each category and the referent group of patients who had not been exposed to alkylating agents. For treatment groups with small numbers of patients, only two dose levels were considered; the median cumulative dose of cytotoxic drug administered to controls was used as the cutpoint. A similar approach was used to examine the relationship between duration of therapy and risk of secondary ANLL. Tests of trend were conducted by assigning the midpoint of the dose or duration of therapy as the representative score for that category, with subsequent entry into a multivariate logistic regression model *(25)*.

The excess risk (excess number of cases) of ANLL among 10 000 NHL patients followed for 10 years was estimated by first multiplying the RR minus 1 by the expected number of ANLL per 10 000 person-years of follow-up, as estimated from our prior cohort study of NHL patients (0.63 ANLL per 10 000 person-years) *(14)*. The product was then multiplied by 8, which is the number of years at risk, assuming a latent period of 2 years before the occurrence of ANLL. For example, a 10-fold risk of ANLL associated with a given alkylating agent would correspond to an excess of 45 ANLLs per 10000 patients over a l0-year period: (10-1) x 0.63 x 8 = 45.

Results

Characteristics of NHL case patients with ANLL and individually matched controls are summarized in Table 1. Men represented approximately 70% of the study group. Most case patients who developed secondary ANLL were aged 50 years or older at the time of NHL diagnosis (median, 54 years; range, 23-70 years). More than one half of the case patients and controls were initially diagnosed with NHL from 1973 through 1980. ANLL occurred an average of 7.6 years after NHL diagnosis (median, 7.5 years; range, 2.4-18.2 years). Case patients with subsequent ANLL were more often treated with radiation and

Table 1. Characteristics of non-Hodgkin's lymphoma patients with secondary acute nonlymphocytic leukemia (case patients) and matched conrrols*

Characteristic	Case patients (n = 35) No.	(%)†	Controls (n = 140) No.	(%)†
Site				
Iowa	4	(11)	16	(11)
The Netherlands	10	(29)	40	(29)
Ontario	13	(37)	52	(37)
Sweden	8	(23)	32	(23)
Sex				
Male	24	(69)	96	(69)
Female	11	(31)	44	(31)
Age at diagnosis of NHL, y				
<50	10	(29)	42	(30)
50-59	12	(34)	48	(34)
60-70	13	(37)	50	(36)
Calendar year of NHL diagnosis				
1965-1972	9	(26)	40	(29)
1973-1980	20	(57)	78	(56)
1981-1989	6	(17)	22	(16)
NHL stage				
I or II	13	(37)	58	(41)
III or IV	14	(40)	44	(31)
Unknown	8	(23)	38	(27)
Latency, y‡				
2-4	10	(29)	40	(29)
5-9	18	(51)	68	(49)
≥10	7	(20)	32	(23)
NHL treatment (all)				
Radiotherapy, no alkylating agents	9	(26)	51	(36)
Alkylating agents, no radiotherapy	9	(26)	31	(22)
Radiotherapy and alkylating agents	17	(49)	46	(33)
Other§	0	(0)	12	(9)

*Matching variables included site, sex, age, calendar year of NHL diagnosis, and latency (please refer to text for detail).

†Percentages may not sum to 100 because of rounding error.

‡Represents interval between diagnosis of NHL and ANLL for case patients and comparable interval for matched controls.

§Includes 10 patients with documented NHL for whom therapy consisted only of surgical resection of involved tissue and two patients treated with nonalkylating agent chemotherapy.

alkylating agents than were controls. Among case patients and controls treated solely with radiotherapy, the median doses of radiation delivered to active bone marrow were 9.3 Gy and 5.1 Gy, respectively. Median doses to active bone marrow for ease patients and controls treated with combined modality therapy including alkylating agents were 15.2 Gy and 8.6 Gy, respectively.

Patients were divided into four chemotherapy groups, based on the major alkylating agent that was administered. These drugs included cyclophosphamide, chlorambucil, prednimustine, and mechlorethamine/procarbazine combinations. The cyclophosphamide treatment group included patients who received this drug either alone or within a cyclophosphamide-based regimen. Patients who were given another alkylating agent in addition to cyclophosphamide were categorized in terms of the other cytotoxic drug because of the weak leukemogenicity of cyclophosphamide compared with other alkylating agents *(2,4,7).*

The number of case patients and controls in each treatment group and data describing cumulative dose of drug and duration of therapy are summarized in Table 2. Eleven case patients and 43 controls received treatment regimens in which cyclophosphamide was the primary alkylating agent. Although cumulative doses of cyclophosphamide were comparable between the two groups, the median duration of therapy was slightly longer among controls (8.8 months) than for case patients (6.8 months). Cyclophosphamide was usually given in combination with vincristine and prednisone (CVP) *(27)* (four case patients and 11 controls) or with vincristine, procarbazine, and prednisone (COPP) *(28)* (three case patients and seven controls), Two case patients and four controls received cyclophosphamide in combination with doxorubicin, teniposide, and prednisone (CHVmP) *(29),* while one case patient and 10 controls received this agent in combination with doxorubicin, vincristine, and prednisone (CHOP) *(30).* Regimens containing the combination of bleomycin, doxorubicin, vincristine, and prednisone (BACOP) were administered to one case patient and three controls *(31).* Other cyclophosphamide-containing regimens were administered to the remaining eight controls.

Chlorambucil comprised the major alkylating agent given to six case patients and 22 controls, with median cumulative doses of 5375 mg and 1003 mg, respectively. Four case patients and 17 controls within this group also received cyclophosphamide, with smaller doses given to case patients (median, 10 950 mg) than to controls (median, 24 000 mg). Median duration of chlorambucil therapy among case patients was approximately three to four times that of controls.

Four patients with secondary ANLL and six controls, all from Sweden, received prednimustine, the C-21 prednisolone ester of chlorambucil. Case patients received more than twice the cumulative dose of this drug than did controls (median doses, 38 020) mg and 18 410 mg, respectively). Within this group, cyclophosphamide was administered to one case patient and two controls at relatively small cumulative doses (10 800 mg to the case patient and 7200 mg and 13 450 mg each to the controls).

Combination chemotherapy regimens containing mechlorethamine, vincristine, procarbazine and prednisone (MOPP) *(32)* were given to four case patients and two controls. Although the median duration of therapy was comparable in both groups, case patients generally received substantially larger doses of both mechlorethamine and procarbazine than did controls.

Multivariate regression techniques were used to estimate the risk of ANLL according to the major alkylating agent that was given (Table 3). Administration of cyclophosphamide as the primary alkylating agent was associated with a nonsignificant 1.8-fold risk of ANLL compared with patients whose treatment did not include alkylating agents. Risks following CHVmP and COPP regimens were somewhat higher than those following CVP, but differences were not significant A 2.4-fold risk of ANLL was observed following chlorambucil-therapy. Significant excesses of ANLL followed treatment with either prednimustine (RR = 13.4) or combination chemotherapy regimens containing both mechlorethamine and procarbazine (RR = 12.6).

Risks of ANLL by categories of cumulative dose and duration of alkylating agent therapy were also estimated with multivariate regression models (Table 4). Increasing cumulative dose

Table 2. Summary of drug dose and duration of administration by treatment group for patients with acute nonlymphocytic leukemia (case patients) and matched controls*

Treatment group	No. of case patients	No. of controls
Cyclophosphamide		
No. of patients	11	43
Dose, mg		
Mean	27 523	20 287
Median	12 150	12 900
Range	3300-179 850	3500-110 150
Duration, mo		
Mean	15.7	15.5
Median	6.8	8.8
Range	2-88	2-153
Chlorambucil†		
No. of patients	6	22
Dose, mg		
Mean	6469	1175
Median	5375	1003
Range	2985-11 634	48-3965
Duration, mo		
Mean	37.3	11.8
Median	32.7	9.2
Range	28-53	2.1-55
Prednimustine‡		
No. of patients	4	6
Dose, mg		
Mean	38 313	25 108
Median	38 020	18 410
Range	10 350-66 860	7720-69 310
Duration, mo		
Mean	30.2	31.8
Median	27.2	20.9
Range	6.8-60	13-67
Mechlorethamine/procarbazine,§ No. of patients	4	2
Mechlorethamine		
Dose, mg		
Mean	195	92
Median	169	92
Range	53-390	46-138
Duration, mo		
Mean	12.6	10.6
Median	11.4	10.6
Range	2.7-25	2.8-18.3
Procarbazine		
Dose, mg		
Mean	15 861	6200
Median	14 172	6200
Range	5950-29 150	2600-9800
Duration, mo		
Mean	12.6	11.3
Median	11.4	11.3
Range	2.7-25	4.3-18.3

*Categories are mutually exclusive based on the primary alkylating agent that was administered and reflect the total chemotherapy history for the patient within the matched time interval at risk. Exposure was defined as treatment with an alkylating agent for more than 1 month. Alkylating agents were usually given in combination with other drugs.

†Four case patients and 17 controls also received treatment with cyclophosphamide; please refer to text for dose.

‡One case patient and two controls also received treatment with cyclophosphamide; please refer to text for dose.

§One case patient also received cyclophosphamide and chlorambucil; another case patient also received CHVmP.

or duration of treatment with cyclophosphamide was not associated with increasing risk of ANLL (*P* for trend = 0.57 and 0.33, respectively). Among patients in the chlorambucil treatment group, elevated risks of ANLL were restricted to patients

Table 3. Risk of acute nonlymphocytic leukemia according to major alkylating agent administered*

Treatment group	No. of case patients	No. of controls	Matched RR†	95% CI
Cyclophosphamide	11	43	1.8	0.7-4.9
Chlorambucil‡	6	22	2.4	0.7-8.6
Prednimustine§	4	6	13.4‖	1.1-156
Mechlorethamine/procarbazine¶	4	2	12.6‖	2.0-79
Other alkylating agents**	1	4	2.9	0.2-41

*Categories are mutually exclusive and reflect the total chemotherapy history for the patient within the matched time interval at risk. Exposure was defined as treatment with an alkylating agent for more than 1 month. Alkylating agents were usually given in combination with other drugs.

†The referent group consisted of nine case patients and 63 controls who were not exposed to alkylating agents.

‡Four case patients and 17 controls also received treatment with cyclophosphamide; please refer to text for dose.

§One case patient and two controls also received treatment with cyclophosphamide; please refer to text for dose.

‖*P*<.05.

¶One case patient also received cyclophosphamide and chlorambucil; another case patient also received CHVmP.

**Includes one case patient who received busulfan, chlorambucil, and cyclophosphamide. Controls (one each) received chlorambucil, lomustine + procarbazine, bleomycin + doxorubicin + cyclophosphamide + vincristine; chlorambucil + prednimustine; chlorambucil, prednimustine + procarbazine; and carmustine + cyclophosphamide.

who received cumulative doses greater than 1300 mg (RR = 6.5) or who were treated for longer than 13 months (RR = 8.3). Excesses of ANLL following prednimustine increased with either increasing cumulative dose or increasing duration of therapy (*P* trend for each <.05), with a 23-fold risk of ANLL evident in the high-dose group. Because only two controls were treated with regimens that included mechlorethamine and procarbazine, cumulative drug dose and duration of therapy were not grouped into categories to evaluate ANLL risk and tests of trend were not conducted.

Treatment with epipodophyllotoxins (10 patients), doxorubicin (37 patients), or bleomycin (10 patients) was not associated with elevated risks of ANLL when adjusted for the effects of alkylating agents in a multivariate model. However, these drugs were typically not given apart from alkylating agents. The median cumulative dose of epipodophyllotoxins administered to case patients and controls was approximately 453 mg and 833 mg, respectively.

The risk of ANLL among patients treated with radiotherapy without alkylating agents was estimated by dividing patients into two groups, baaed on the median dose of radiation delivered to active bone marrow among controls. With this approach, the RR of ANLL among patients given high-dose radiation (6.35 Gy or more; six case patients and 22 controls) was 3.1 (*P* = .13) compared with patients who received either radiation or low-dose radiation (<6.35 Gy; three case patients and 41 controls). The extent of radiotherapy (i.e., proportion of bone marrow irradiated) did not influence the subsequent risk of ANLL when adjusted for radiation dose. The risk of ANLL among patients treated with combined modality therapy did not differ from the risk among those given alkylating agents alone when adjusted for drug dose and type.

9000-22 000 mg	12 525 mg	4	14	2.1	0.5-8.7
>22 000 mg	31 510 mg	3	15	1.4§	0.3-5.7
Duration					
<6 mo	4.5 mo	4	14	2.0	0.5-8.6
6-14 mo	8.2 mo	3	14	1.5	0.4-6.6
>14 mo	18.7 mo	4	15	1.9‖	0.5-7.4
Chlorambucil‡¶					
Dose					
<800 mg	428 mg	0	8	0	—
800-1300 mg	1015 mg	0	7	0	—
>1300 mg	3164 mg	6	7	6.5**	1.6-26
Duration					
<5 mo	4.3 mo	0	7	0	—
5-13 mo	8.6 mo	0	7	0	—
>13 mo	24.6 mo	6	8	8.3**	1.8-38
Prednimustine†††‡					
Dose					
<18 410 mg	12 475 mg	1	3	6.8	0.3-134
≥18 410 mg	38 020 mg	3	3	23.1§§	1.4-380
Duration					
<20.9 mo	15.3 mo	1	3	7.2	0.3-149
≥20.9 mo	39.5 mo	3	3	20.5§§	1.3-333

*Categories are mutually exclusive and reflect the total chemotheraphy history for the patient within the matched time interval at risk. Exposure was defined as treatment with an alkylating agent for more than 1 month. Alkylating agents were usually given in combination with other drugs.
†The referent group consisted of nine case patients and 63 controls who were not exposed to alkylating agents.
‡Categories are based on tertiles of cumulative dose or duration of therapy among controls.
§Test for trend, $P = .57$.
‖Test for trend, $P = .33$.
¶Four case patients and 17 controls also received cyclophosphamide; please refer to text for dose.

**Although test for trend is significant ($P<.05$), increased risk is restricted to patients treated with cumulative dose >1300 mg or duration >13 months.
††One case patient and two controls also received cyclophosphamide; please refer to text for dose.
‡‡Categories are based on the median value for cumulative dose or duration of therapy among controls.
§§Test for trend, $P<.05$.

Risk of ANLL following alkylating agent therapy did not vary significantly by age at NHL diagnosis when evaluated by multivariate methods, although patients aged 60 years or older demonstrated increased risks (RR = 3.6; 95% CI = 1.0-13) compared with those aged 50-59 years (RR = 1.8) or younger than 50 years (RR = 2.7). Using multivariate techniques, excesses of ANLL following alkylating agent therapy were comparable for men and women. NHL stage was not related to subsequent risk of ANLL when adjusted for the effect of alkylating agents.

Twofold risks for secondary ANLL followed alkylating agent therapy in each study site, except in Sweden where sixfold risks (largely attributable to the use of prednimustine) were observed There were no significant differences, however, between sites.

Discussion

This study is one of the few investigations of secondary ANLL among NHL patients that includes information on cumulative dose for all administered alkylating agents as well as estimates of radiation dose to bone marrow for individual patients. New observations include quantification of the carcinogenicity of prednimustine and additional assessment of the dose-response relationship between chlorambucil and secondary ANLL. We also provide further data on the leukemogenicity of low doses of cyclophosphamide, an alkylating agent widely used in numerous cytotoxic and immunosuppressive regimens.

One provocative finding in this investigation is the significantly increased risk of secondary ANLL following prednimustine therapy, with evidence of a dose-response gradient. Very little data exist regarding the human carcinogenicity of prednimustine *(33)*, although it has been used as both primary *(34,35)* and secondary *(36,37)* therapy for NHL and chronic lymphocytic leukemia as well as in the treatment of breast cancer *(38-40)*. An elevated risk of secondary ANLL, based on two cases, was recently reported among women with advanced breast cancer who were treated with combination chemotherapy regimens that included prednimustine *(40)*. Our study is the first to suggest that a dose-response relationship may exist between the cumulative amount of prednimustine and secondary ANLL and is additional evidence of the potential carcinogenicity of this agent.

There are few quantitative descriptions of the leukemogenicity of chlorambucil *(4,41,42)*. The initial report linking this alkylating agent with excesses of secondary ANLL *(41)* provided risk estimates stratified by the average daily dose of chlorambucil given to patients with polycythemia vera. Data on the number of days of administration or the cumulative dose of chlorambucil, however, were not provided, and it is unclear if

such patients might be at high natural risk of leukemia because of their underlying disorder. A 160-fold risk of ANLL following chlorambucil therapy for ovarian cancer was reported by Greene et al. *(42)*, based on two case patients who received cumulative doses of more than 2000 mg. More recently, Kaldor et al. *(4)* found that both low (median, 170 mg) and high (median, 3200 mg) cumulative doses of chlorambucil administered to women with ovarian cancer were associated with significantly elevated risks of leukemia. In contrast, elevated risks for ANLL in our study were evident among only those patients in the highest tertile of cumulative dose. Further quantification of the dose-response relationship between chlorambucil and secondary leukemia is needed, given that this drug [which is a known leukemogen *(4,41-44)]* remains part of the standard initial treatment regimen for chronic lymphocytic leukemia *(45)*.

Administration of cyclophosphamide as the primary alkylating agent in various combination chemotherapy regimens was associated with considerably smaller risks of ANLL in our study than was treatment with either prednimustine or mechlorethamine and procarbazine. These results are consistent with prior reports *(2,4,7)*, which indicate that cyclophosphamide is a relatively weak leukemogen compared with other alkylating agents, Only a few investigations *(2-4,7,12)*, however, have included quantification of the relationship between cyclophosphamide dose and risk of ANLL. The small excesses of ANLL apparent at cumulative cyclophosphamide doses of less than 20 000 mg in our study are consistent with findings among patients with breast cancer *(3,7)* or ovarian cancer *(2,4)*, in whom nonsignificant risks of ANLL accompanied low doses of this drug.

A dose-response gradient between cyclophosphamide dose and risk of ANLL was not apparent in our investigation. Elevated risks for ANLL, however, have been reported in other studies *(2-4,7,12)* following large cumulative doses of cyclophosphamide. Thus, the carcinogenic potential of cyclophosphamide should not be discounted, although current evidence suggests that smaller cumulative doses incur lower risks of ANLL. The lack of significance in prior low-dose studies is probably due to the small numbers evaluated and the associated reduction in statistical power. Additional characterization of the relationship between cyclophosphamide dose and secondary ANLL seems warranted, given the critical role of this agent in various antineoplastic and immunosuppressive treatment regimens *(46-48)*. Moreover, cyclophosphamide-containing regimens are now increasingly used as adjuvant therapy in early-stage NHL *(49)*.

Mechlorethamine and procarbazine constitute part of the well-known MOPP combination chemotherapy regimen *(32)*, which provided a critical advance in the treatment of malignant lymphoma. Our results in NHL patients confirm the leukemogenicity of MOPP that has been reported following Hodgkin's disease *(5,50-52)*. Previous studies *(5,51)* have found that ANLL risk tends to increase with increasing number of cycles of MOPP; however, few studies have evaluated the risk associated with cumulative dose of the cytotoxic drugs that comprise the regimen *(51)*, In one investigation *(51)* the average cumulative dose of mechlorethamine given to Hodgkin's disease patients with subsequent ANLL was 77.5 mg/m^2, with control subjects receiving 64.6 mg/m^2. Doses in our study were expressed in terms of cumulative dose, with case patients receiving approximately twice the amount of mechlorethamine given to controls. Evaluation of the human carcinogenicity of procarbazine, another component of MOPP, remains elusive *(53)*, since this drug is usually given in combination with mechlorethamine. However, an elevated risk of ANLL was reported among Hodgkin's disease patients treated with more than six cycles of combination chemotherapy that included procarbazine and cyclophosphamide *(5)*. This finding, based on three case patients and one control, remains to be confirmed in other investigations.

Kaldor et al. *(5)* reported that the leukemogenicity of MOPP among patients with Hodgkin's disease does not appear to be affected by concomitant radiotherapy, whereas other investigators have suggested that it may be increased by extended-field irradiation *(51,54)*. In general, the evidence is conflicting with regard to the relative leukemogenicity of combined modality therapy compared with chemotherapy alone in the treatment of Hodgkin's disease *(16,54-56)* and other cancers *(2,4,7)*. Combined modality therapy using involved-field radiotherapy to treat NHL may not enhance the risk of ANLL associated with alkylating agents *(10,13)*. However, when combined modality therapy that incorporates extended-field radiation is used to treat NHL, significantly greater risks of ANLL may result than when following single modality therapy *(12)*. Although the results of several analytic approaches in our study suggested that radiotherapy did not add to the leukemogenicity of alkylating agents, cautious interpretation of these findings is advised because of the small number of patients and the large number of parameters evaluated.

In general, our results should be viewed in light of several strengths and limitations of this internationally based study. The strong points of our investigation include the near-complete ascertainment of chemotherapy and radiotherapy information, the histologic confirmation of ANLL diagnoses, the large numbers of NHL patients available for study, and the ability to evaluate treatment regimens in current use today. The main limitation is the small number of ANLL cases (only 35), which restricts the inferences that can be drawn when subgroup analyses are conducted and interaction effects are evaluated. Nonetheless, our results indicate that treatment of NHL with any of several standard alkylating agents incurs a measurable risk of subsequent ANLL, with the magnitude of the excess dependent upon the specific drug that is used and the cumulative dose. On the basis of our earlier cohort study *(14)*, 10 000 NHL patients treated for 6 months with selected chemotherapy regimens that include low cumulative doses of cyclophosphamide and who are followed for 10 years might develop an excess of four ANLLs. If MOPP or prednimustine-based regimens similar to those in our study were administered, up to 62 excess ANLLs might result. As always, the therapeutic efficacy of various treatment regimens must be carefully weighed against the associated risk of secondary leukemia. Even then, the significant improvements in patient survival afforded by a variety of treatment regimens for NHL far outweigh the associated small excess risk of secondary ANLL.

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Notes

We thank the many hospitals and physician who allowed access to treatment records; Virginia Hunter, Loretta Brown, Alexandra van den Belt-Dusebout, Robert Oppliger, and Nancy Holt for support in data collection; Diana Wagner and Carla Van Hoesen for assistance in identifying eligible cases; Sara Brackett for technical coordination of the histopathologic review; Diane Fuchs for administrative assistance in conducting the field studies; George Giese and Roberta McClimens for help with database management; Rebecca Albert for technical support and Sandy Coopersmith for secretarial assistance.

Manuscript received March 25, 1994; revised June 28, 1994; accepted July 6, 1994.